

WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding an enzyme having astaxanthin synthase activity which catalyzes the reaction of beta-carotene to astaxanthin.
- 5 2. An isolated polynucleotide according to claim 1 wherein the reaction is catalyzed in *P. rhodozyma*.
3. An isolated polynucleotide according to claim 1 which is selected from
- (a) a nucleotide sequence which encodes an enzyme having the amino acid sequence shown in SEQ ID NO: 1, or
- 10 (b) a nucleotide sequence which encodes (i) an allelic variant of SEQ ID NO:1 or (ii) an enzyme having the polypeptide sequence of SEQ ID NO:1 with one or more amino acid insertions, deletions, and/or substitutions, which variant has astaxanthin synthase activity.
- 15 3. An isolated polynucleotide according to claim 1 which is selected from the group consisting of:
- (i) SEQ ID NO: 2;
- (ii) a nucleotide sequence which, because of the degeneracy of the genetic code, encodes an astaxanthin synthase having the same amino acid sequence as that encoded
- 20 by SEQ ID NO:2; and
- (iii) a nucleotide sequence which hybridizes to the complement of the nucleotide sequence from i) or ii) under standard hybridizing conditions (50% v/v formamide, 5X SSC, 2% w/v blocking agent, 0.1% N-lauroylsarcosine, 0.3% SDS at 42EC overnight).
- 25 4. An isolated polynucleotide according to claim 1 which is selected from the group consisting of:
- (i) SEQ ID NO: 3;
- (ii) a nucleotide sequence which, because of the degeneracy of the genetic code, encodes an astaxanthin synthase having the same amino acid sequence as that encoded
- 30 by SEQ ID NO:3; and

(iii) a nucleotide sequence which hybridizes to the complement of the nucleotide sequence from i) or ii) under standard hybridizing conditions (50% v/v formamide, 5X SSC, 2% w/v blocking agent, 0.1% N-lauroylsarcosine, 0.3% SDS at 42EC overnight).

5. A vector or plasmid comprising a polynucleotide which encodes an enzyme having astaxanthin synthase activity.

6. A vector or plasmid according to claim 5 wherein the polynucleotide encodes SEQ ID NO:1.

10

7. A vector or plasmid according to claim 5 wherein the polynucleotide is SEQ ID NO:2.

15

8. A vector or plasmid according to claim 5 wherein the polynucleotide is SEQ ID NO:3.

9. A host cell transformed or transfected with a polynucleotide which encodes an enzyme having astaxanthin synthase activity.

10. A host cell according to claim 9 wherein the polynucleotide encodes a polypeptide having the sequence of SEQ ID NO:1.

11. A host cell according to claim 9 wherein the polynucleotide is SEQ ID NO:2.

12. A host cell according to claim 9 wherein the polynucleotide is SEQ ID NO:3.

13. A host cell according to claim 9 which is transfected or transformed with a vector or a plasmid comprising: (a) a polynucleotide which encodes the polypeptide of SEQ ID NO:1; (b) SEQ ID NO:2; and/or (c) SEQ ID NO:3.

30

14. A recombinantly-produced polypeptide encoded by a polynucleotide which is SEQ

ID NO:2 or SEQ ID NO:3.

16 15. A process for producing a polypeptide having astaxanthin synthase activity comprising culturing a host cell transformed with a polynucleotide which encodes an enzyme having astaxanthin synthase activity under conditions conducive to produce the enzyme.

17 16. A process according to claim 15 wherein the polynucleotide encodes the polypeptide of SEQ ID NO:1.

10

18 17. A process according to claim 15 wherein the polynucleotide is SEQ ID NO:2.

19 18. A process according to claim 15 wherein the polynucleotide is SEQ ID NO:3.

15 20 19. A process for producing astaxanthin comprising introducing one or more isolated polynucleotides encoding an enzyme having astaxanthin synthase activity into an appropriate host organism; cultivating the organism under conditions conducive to produce astaxanthin; and recovering astaxanthin from the culture.

20 21 20. A process according to claim 19 wherein the polynucleotide is selected from the group consisting of a polynucleotide encoding SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

22 21. A process for producing astaxanthin comprising contacting beta-carotene with a polypeptide having astaxanthin synthase activity in the presence of an electron donor in a reaction mixture containing a reconstituted membrane.

23 22. A process according to claim 21, wherein the polypeptide is present in the form of a reconstituted membrane which is prepared from a biological membrane.

30

24 23. A process according to claim 22 wherein the membrane is a microsome or a

mitochondrial membrane.

23 24. A process according to claim 21 wherein the polypeptide is present in the form of a reconstituted artificial membrane.

5

24 25. A process according to claim 24 wherein the reconstituted artificial membrane is a liposome.

25 26. A process according to claim 21, wherein the electron donor reduces a reaction center of the astaxanthin synthase.

10

26 27. A process according to claim 26 wherein the electron donor is cytochrome P450 reductase

15 28. An isolated polynucleotide encoding a polypeptide which is SEQ ID NO:1.

20 29. An isolated polynucleotide consisting of SEQ ID NO:2.

30 30. An isolated polynucleotide consisting of SEQ ID NO:3.

20

31 31. A recombinantly-produced polypeptide having astaxanthin synthase activity which is SEQ ID NO:1.

32 32. A vector comprising a polynucleotide selected from the group consisting of a polynucleotide which encodes SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

25

33 33. A host cell transformed with the vector of claim 32.

34 34. A process for producing astaxanthin comprising: (a) cultivating in a suitable culture medium a recombinantly produced host cell containing a polynucleotide which encodes a polypeptide having astaxanthin synthase activity.

30

35. A process according to claim 34 wherein the polynucleotide encodes a polypeptide which is SEQ ID NO:1.

36. A process according to claim 34 wherein the polynucleotide is SEQ ID NO:2.

37. A process according to claim 34 wherein the polynucleotide is SEQ ID NO:3.

38. A process according to claim 34 further comprising isolating the astaxanthin synthase from the host cell or the culture medium.

10066007, 026102